PROCESS CONSIDERATIONS IN CRUDE OIL BIODESULFURIZATION

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ABSTRACT

Biodesulfurization offers an attractive alternative to conventional hydrodesulfurization due to the mild operating conditions and reaction specificity afforded by the biocatalyst. The enzymatic pathway existing in *Rhodococcus* has been demonstrated to oxidatively desulfurize the organic sulfur occurring in dibenzothiophene while leaving the hydrocarbon intact. In order for biodesulfurization to realize commercial success, a variety of process considerations must be addressed including reaction rate, emulsion formation and breakage, biocatalyst recovery, and both gas and liquid mass transport. This study compares batch stirred to electro-spray bioreactors in the biodesulfurization of both model organics and actual crudes using a *Rhodococcus* IGTS8 biocatalyst in terms of their operating costs, ability to make and break emulsions, ability to effect efficient reaction rates and enhance mass transport. Additionally, sulfur speciation in crude oil is assessed with respect to sulfur specificity of currently available biocatalysts.

KEY WORDS: crude oil desulfurization, *Rhodococcus*, electrostatic spraying, dibenzothiophene, biodesulfurization

INTRODUCTION

Biological processing of fossil fuel feedstocks offers an attractive alternative to conventional thermochemical treatment due to the mild operating conditions and greater reaction specificity afforded by the nature of biocatalysis. Efforts in microbial screening and development have identified microorganisms capable of petroleum desulfurization (see for example, [1-3]), denitrification [4], demetalization [4], cracking [5] and dewaxing. Biological desulfurization of petroleum may occur either oxidatively or reductively. In the oxidative approach, organic sulfur is converted to sulfate and may be removed in process water. This route is attractive due to the fact that it would not require further processing of the sulfur and may be amenable for use at the well head where process water may then be reinjected. In the reductive desulfurization scheme, organic sulfur is converted into hydrogen sulfide, which may then be catalytically converted into elemental sulfur, an approach of utility at the refinery. Regardless of the mode of biodesulfurization, key factors affecting the economic viability of such processes are biocatalyst activity and cost, differential in product selling price, sale or disposal of co-products or wastes from the treatment process, and the capital and operating costs of unit operations in the treatment scheme.

In all fossil fuel bioprocessing schemes, there is a need to contact a biocatalyst containing aqueous phase with an immiscible or partially miscible organic substrate. Factors such as liquid / liquid and gas / liquid mass transport, amenability for continuous operation and high throughput, capital and operating costs, as well as ability for biocatalyst recovery and emulsion breaking are significant issues in the selection of a reactor for aqueous / organic contacting. Traditionally, impeller-based stirred reactors are utilized for such mixing due to their ease of operation and wide acceptance in the chemical and biological processing industries. Such mechanically stirred reactors contact the aqueous and organic phases by imparting energy to the entire bulk solution, i.e. the impeller must move the contents of the reactor.

Recent advances in the area of contactors for solvent extraction have lead to the development of electrically driven emulsion phase contactors (EPC™) for efficient contact of immiscible phases [6]. In this concept, the differing electrical conductivity between the aqueous and organic phases causes electrical forces to be focused at the liquid / liquid interface, creating tremendous shear force. This shear causes the conductive phase to be dispersed (5 □m droplet size) into the non-conductive phase, but does so with decreased energy requirements relative to mechanical agitators due to the fact that energy is imparted only at the liquid / liquid interface and not the entire bulk solution. In a configuration of the EPC™ developed at the Oak Ridge National Laboratory, the contactor serves to disperse aqueous phase containing biocatalyst into an organic

phase. The EPC™ creates droplets of water containing biocatalyst ~5 □m in diameter within an organic phase.

Here, we compare the performance of the EPC™ to that of a batch stirred reactor (BSR), investigate the required level of biocatalyst activity before the surface area afforded by the EPC™ becomes a factor in reactor performance, and characterize the emulsion formed by both reactors in the presence of bacteria. We have investigated the emulsion quality formed in the EPC, evaluated the power requirements and analyzed the mass transfer issues in comparison to stirred reactors. Results on biodesulfurization of actual crude oil by wild type *Rhodococcus* IGTS8 are also included. Finally, we assess the sulfur specificity of available biocatalysts with respect to sulfur compounds present in crude oils.

MATERIALS AND METHODS

The experimental procedures used for studying biodesulfurization in model systems have been discussed in detail in previous publications [7-9]. A detailed description of oil experiments is provided here.

Biodesulfurization of Van Texas Crude oil

Biodesulfurization of Van Texas crude oil was studied in batch stirred reactors to evaluate the substrate specificity of the biocatalyst. The experiment was conducted over a treatment period of 6 days. The crude had an API specific gravity of 31°, and a sulfur content of 0.96 wt.%. The crude oil did not contain volatiles due to production at elevated temperature (~99°C). Experiments were performed in batch stirred reactors utilizing 50 g of frozen *Rhodococcus sp.* wild type strain IGTS8 (ATCC 53968) cell paste which were brought up to 750 mL with 0.156M (pH 7.5) phosphate buffer. The cells were suspended in the phosphate buffer prior to addition to the reactor. The reactor vessel used was a 1-L VirTis Omni-Culture fermentor (model 178657, Gardiner, NY), utilizing a 6-bladed Rushton-type impeller with 2 baffles. The reactor was kept at 30°C, agitated at 800 RPM, and aerated with room air at a rate of 0.2 standard liters per minute (SLPM). A water condenser was used on each reactor to capture volatiles which were expected to be minimal or non-existent considering the fact that the operating temperature was much less than that of the oil reservoir. The experiment was conducted with 250 mL of crude oil, treated with 750 mL of the aqueous phase. Samples (30 mL from the top of the organic phase) taken during the course of biological treatment were collected after ceasing the agitation and aeration for 5 min to allow the aqueous and organic phases to separate. The reactor contents were emptied at the end of the run and centrifuged at 6000 rpm in a Beckman Model TJ-6 centrifuge to obtain a sample of treated crude oil. Closed samples were boiled in a closed container for 30 min to halt biological activity.

Analytical

Model system experiments

In the experiments reported here, DBT and 2-HBP concentrations in the aqueous phase were below our levels of detection. DBT and 2-hydroxybiphenyl (2-HBP) concentrations in n-hexadecane were measured by gas chromatography using a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector.

Crude oil

A GC-SCD method was used to determine the sulfur content of the aromatic fraction of the oil. To allow facilitated observation of sulfur in the treated oil, whole oil samples were fractionated according to ASTM method D2007. An extended ASTM D2887 procedure was used for chromatographic separation of the aromatic fraction of the crude oil. Sulfur analysis was performed by modifying the ASTM D2887 procedure by adding a Sievers Chemiluminesence sulfur specific detector after the flame ionization detector.

RESULTS AND DISCUSSION

Rate of biodesulfurization

The specific rate of DBT desulfurization by *Rhodococcus sp.* was typically between 1 and 5 mg 2-HBP produced per dry g of biocatalyst per hour. Specific rates of 2-HBP production in the batch stirred reactor and the EPC™ reactor systems were within experimental variance and no

appreciable difference in desulfurization rates were seen between the two reactors. Due to the high surface area reported in the EPC™ [7], higher rates were expected in the EPC™, however, similar performance was observed in both reactors. The reaction rate obtained was without any supplemental carbon or energy source. Note that the only available carbon and energy source for the biocatalyst other than what may be carried over in the frozen cell paste, was hexadecane and DBT. However, DBT was not used as the carbon source by the biocatalyst, since the end product of DBT conversion was 2-HBP (thus preserving the carbon number and fuel value). Other studies (outlined in [7]) have utilized additional external carbon and energy sources and have reported higher activities with *Rhodococcus sp.* A commercial scale biodesulfurization process may require a higher cell density to achieve maximum conversion in a minimum time, provided it does not affect yield with respect to biocatalyst usage. In order to study effect of cell density, experiments were conducted in the BSR at different cell loadings. This experiment also lead to determination of the cell density at which point the BSR becomes mass transfer limited. A following experiment in the EPC™ at this cell density was conducted to evaluate the benefits of high surface area afforded by the EPC™.

Mass transport issues

The rate of desulfurization, when normalized with respect to cell mass, was found to decrease with increasing cell density indicating that mass transfer resistance was the controlling process in desulfurization. A statistical analysis of the data indicated mass transfer limitation between 5X and 10X cell density in the BSR. The mass transfer limitation may be due to gas-liquid or liquid-liquid mass transport resistance.

The results of experiments conducted in the BSR at 10X cell density indicated no gas-liquid mass transfer limitation. Increasing the rate of air supply or increasing the oxygen tension in the reactor through the use of pure oxygen rather than air did not seem to affect HBP production. This suggests that the system may be limited by liquid-liquid mass transfer. Since the EPC™ reportedly provides larger liquid-liquid interfacial area, the BSR was compared with the EPC™ for desulfurization activity at the high cell density.

Comparison of the EPC™ and BSR performance at 10X cell density showed no difference in the desulfurization rates between the two reactors. Thus, either the system is not truly mass transport limited or the EPC™ did not provide a larger surface area for reaction under the present conditions. A detailed characterization of the emulsions formed in the BSR and EPC™ in the presence and absence of biocatalyst was conducted and is reported below.

Emulsion quality in BSR and EPC™

A detailed drop size analysis of the two-phase emulsion formed in the BSR has been reported previously [7]. Characterization of the emulsion quality in BSR in the absence of biocatalyst has revealed 100-200 micron droplets under the conditions of experiments conducted here. The droplets formed in the EPC; however, are in the 1-10 micron range. The ability to form fine emulsions in the EPC™ without increasing energy utilization (see energy utilization section below) could have tremendous impact upon processing costs assuming that the biocatalyst utilized is active enough to be mass transport limited.

Emulsion quality in the presence of biocatalyst

Due to the opaqueness rendered by presence of biocatalyst, observations could not be made *in situ* during reactor operation. To determine the emulsion quality formed in the EPC™ and BSR, and to determine whether the EPC™ offers larger surface area than BSR, samples were collected from the reactors and observed under a microscope using a 100x oil emersion objective. Microscopic examination of samples showed formation of a very fine emulsion in both reactors with droplet sizes ranging from 1 to 10 $\square$m. Formation of such an emulsion in the BSR may be presumed due to production of biosurfactants by the biocatalyst IGTS8. Average droplet size for EPC™ and BSR samples were 2.54 ± 2.40 $\square$m and 3.08 ± 1.78 $\square$m, respectively (n>300). Further, a significant amount of the biocatalyst was extracted and existed in the organic phase. Thus, a very fine emulsion is formed in the EPC™ as well as the BSR, and it appears that it is for this reason that an augmentation in desulfurization rate is not seen in the EPC™ relative to the BSR. A couple of process issues warrant consideration here. Firstly, due to the formation of a fine stable emulsion, downstream separation of the multiphase mixture to obtain clean organic fuel may require additional separation processes. Secondly, the *Rhodococcus* biocatalyst used in these experiments was extracted into the organic phase. If biocatalyst recovery and reuse is desired, separation of the biocatalyst from aqueous as well as organic phases will be required.

Energy utilization by EPC™

Typically, stirred reactors or impeller based reactors are capable of achieving water or oil droplet sizes of 100 -300 □m in diameter under the conditions used in this study when surfactants are not present. Tin order to create such droplet size distribution, the energy required is on the order of 1-6 W/L (based upon empirical correlation's [10]). It is estimated that if impeller based systems were capable of producing 5 □m droplets, it would require ~25 kW/L [11] if surfactants are not present. The EPC™ creates droplets of water containing biocatalyst ~5 □m in diameter within an organic phase, and does so with a power requirement of 3 W/L [7]. Thus, if a high activity biocatalyst is available, which is actually limited by mass transport, the EPC™ could result in tremendous savings over the batch stirred reactor. For instance, on a 1 L basis, a BSR using a 3:1 water to oil ratio and producing oil droplets of 150 mm in diameter creates 1×10^5 cm^2 of interfacial surface area. On the same volume basis, an EPC™ creating 5 □m diameter aqueous droplets and having a 5% aqueous hold-up creates 6×10^5 of interfacial area at 1/15th the aqueous volume to do so. In a mass transport system, the rate of desulfurization would thus be expected to be six times as large using 93% less biocatalyst. An additional important point which needs to be noted here is that the fine emulsion formed in the EPC™ is an unstable emulsion i.e., the emulsion breaks easily upon removal of the electric fields giving easy separation of the organic and aqueous streams.

Crude oil biodesulfurization

Oil samples collected from the BSR were analyzed by GC-SCD to obtain the distribution of organosulfur compounds in the crude oil. Analysis was done on the aromatic fraction of the oil and not the whole oil, since the baseline did not return to its initial value in case of the whole oil. This fraction of the Van Texas oil accounted for 22% of the oil's original volume. As shown in Figure 1, the total sulfur content of this aromatic fraction was reduced from 3.8 to 3.2% in 6 days of treatment with IGTS8.

The results indicate removal of comparatively low molecular aromatic sulfur compounds; however, a large portion of the organosulfur fraction does not seem to be affected by the biodesulfurization process. Additional analysis by GC-MS (not shown here) has revealed up to 90% sulfur removal from DBT and substituted DBT compounds. While it appears that this biocatalyst is capable of desulfurizing the majority of sulfur species present in diesel (DBT and substituted DBT compounds) and that only improvements in the rate of desulfurization are needed for the commercialization of this process, a great deal of research is needed for oil biodesulfurization to be realized. The sulfur specific oxidation of DBT by *Rhodococcus* resulted from over 15 years of research using DBT as the model organic sulfur compound in coal and oil. Detailed sulfur speciation studies and biocatalyst development is needed to achieve desulfurization of the broad spectrum of organic sulfur species present in crude oil and to realize the promises of petroleum biodesulfurization.

CONCLUSIONS

A variety of process considerations in the biodesulfurization of petroleum feedstocks were addressed in this study including reaction rate, emulsion formation and breakage, biocatalyst recovery, and both gas and liquid mass transport. Comparison of batch stirred reactor to EPC™ revealed formation of high surface area in the EPC™ in the absence of surface-active agents. Presence of biocatalysts capable of producing biosurfactants results in fine emulsions in both reactors; however, poses a potentially more difficult problem with downstream multiphase separation. The use of EPC™ as a biodesulfurization reactor can result in up to several orders of magnitude energy savings over BSR in the absence of surfactants. Gas-liquid mass transfer was not a limiting factor in biodesulfurization studies with model systems. Further, biodesulfurization experiments with actual crude oil showed that presently available biocatalysts such as *Rhodococcus* sp. IGTS8 are capable of removing DBT and substituted DBT type compounds but do not affect the remaining portion of the organosulfur compounds. Thus, there is a need for further development in biocatalysts capable of desulfurization of higher molecular weight non-DBT type sulfur compounds present in crude oil.

ACKNOWLEDGMENTS

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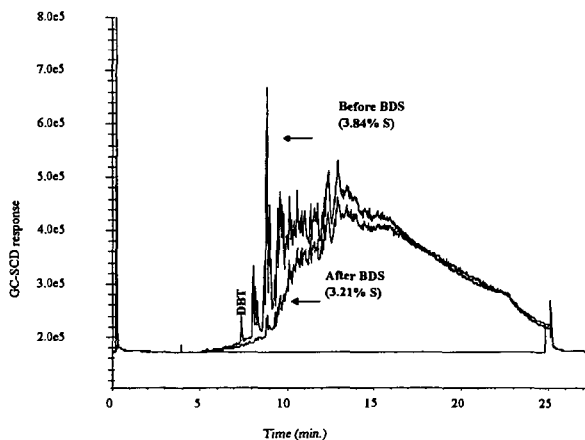
Figure 1. Analysis of the aromatic fraction of Van Texas crude oil by GC-SCD. The biotreatment results in removal of low molecular weight DBT-type compounds; however, the higher molecular weight compounds are not affected.